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Postprandial concentrations of free and conjugated bile acids down the length of the normal human small intestine

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SUMMARY Small intestinal samples were obtained by intubation from multiple sites along the small intestine in 11 subjects with no known gastrointestinal disease eating a normal diet and at laparotomy in a further three subjects. Free (unconjugated) bile acids were consistently demonstrated in ileal samples, and occasionally in lower jejunal samples, by thin-layer chromatography, supplemented in some cases by gas/liquid chromatography and by infrared spectroscopy. The free bile acid concentration, measured enzymically following thin-layer chromatography, reached a maximum (1 mM) in the lower ileum, where it represented half the total bile acid concentration. Following ampicillin, the concentration of free bile acids decreased markedly, suggesting that they resulted from bacterial deconjugation; at the same time the total bile acid concentration increased, suggesting impaired absorption due to the reduced concentration of the more rapidly absorbed free bile acids. Our results indicate that the presence of free bile acids in lower jejunal and ileal samples is a normal finding, and cannot be taken as evidence of abnormal bacterial overgrowth. They also suggest that bacterial deconjugation at these sites may be a factor contributing to the remarkable efficiency of bile salt reabsorption.

The presence of free bile acids has been reported in small intestinal content from patients with the stagnant loop syndrome, where they result from bacterial deconjugation (Donaldson, 1965; Tabaqchali and Booth, 1966). In most patients with this syndrome, free bile acids are found at all levels of the small intestine, but in patients with an ileal stricture they may be restricted to the ileum (Tabaqchali, Hatzioannou, and Booth, 1968). Current evidence suggests that bile acid deconjugation is the cause of steatorrhoea in the stagnant loop syndrome, either as a direct toxic effect of the free bile acids (Dawson and Isselbacher, 1960; Donaldson, 1965) or as a result of a reduction in the concentration of conjugated bile acids to a level inadequate for micellar solubilization of dietary fat (Tabaqchali *et al*, 1968).

Altered bile acid metabolism in patients with the stagnant loop syndrome has been evaluated on the assumption that bacterial deconjugation of bile acids does not occur in the normal small intestine, but adequate control data are lacking. Sjövall (1959) analysed samples from the distal and proximal small intestine in two normal subjects, using paper chromatography without any prior extraction procedure. He found a similar bile acid composition at both sites, with practically all the bile acids in the conjugated form. Using more recently developed analytical techniques, we have consistently demonstrated the presence of free (unconjugated) bile acids in distal small intestinal samples from subjects with no known gastrointestinal disease. We have also determined normal values for the concentrations of free and of conjugated bile acids down the length of the small intestine during ingestion of a normal diet. These supplement figures for total bile acid concentration at different levels of the small intestine in 10 normal subjects, previously published by Fordtran and Locklear (1966). A preliminary report of our findings was presented at the annual meeting of the British Society of Gastroenterology in 1970 (Northfield, Condillac, and McColl, 1970).

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Material and Methods

Intubation studies were carried out on 11 inpatients. None of the patients had any known gastrointestinal disease and none of them had gastrointestinal symptoms. The patients were convalescing after a myocardial infarct in two cases, after surgery for pilonidal sinus in two cases, and after surgery for varicose veins in two further cases. Other pathologies included idiopathic cardiac arrhythmia (1), carcinoma of bronchus (1), diabetes mellitus (1), peripheral vascular disease (1), and deep vein thrombosis (1). The general physical state of all the patients was good at the time of the investigation. None of them had had any antibiotics in the previous month, nor any other drugs apart from night sedation during the previous week. Their ages ranged from 21 to 66 (mean 47 years). Ten subjects were male, one female.

Samples were obtained by means of a modification of the rapid transit tube of Wiggins, Cook, and McLeod (1967). The triple-lumen tube was passed via the nasal route before breakfast on the day of the study, following an overnight fast. Its passage into the duodenum was checked radiologically, and further passage was assessed by measuring the length of tube protruding from the nose. A fasting sample was obtained and the subject was given a normal ward breakfast. The balloon was inflated and further postprandial samples were obtained at intervals down the small intestine. The postprandial upper jejunal sample was obtained approximately 30 minutes after breakfast, and all other samples were obtained within two hours of eating. The subject continued meanwhile to eat a normal ward diet of three full meals daily. In five subjects the tip of the tube reached a position within 18 inches of the ileocaecal valve, as demonstrated radiologically, within a period of 12 to 36 hours after its introduction. The samples obtained were regarded as coming from five equal segments of jejunum and ileum (upper and lower jejunum, and upper, mid, and lower ileum) on the basis of measurements of tube length, combined with radiological checking of position at the duodenojejunal flexure and close to the ileocaecal valve. In the six patients in whom the tube did not reach the lower ileum, the segments were based on an assumed length of 90 inches for jejunum and ileum, this being the mean length in the other five subjects. An additional single sample was obtained by needle aspiration from three further subjects at laparotomy, the site being determined by direct inspection. None of these three subjects was suspected of having any small intestinal pathology, and the small intestine appeared normal on inspection. The period of fasting before surgery was approximately four hours in each case.

All samples obtained were immediately frozen at −10°C. At the time of analysis, they were brought to room temperature, an aliquot was evaporated to dryness, and the residue extracted three times with redistilled methanol. This extraction procedure gives recoveries of 75 to 80% for free bile acids and of 85 to 90% for conjugated bile acids.

Qualitative analysis was carried out on each sample by thin-layer chromatography on plates spread with Kieselgel G, activated for one hour at 110°C immediately before use. For identification of free bile acids, the solvent systems S7 and S11 of Eneroth (1963) were used (benzene, isopropyl alcohol, and acetic acid 30:10:1 and trimethyl pentane, ethyl acetate, acetic acid 10:2:2 by volume respectively), and for conjugated bile acids the solvent system of Anderson and Haslewood (1970) was used (amyl acetate, acetic acid, water 7·5: 7·5: 3·0 by volume). Plates were developed with 10% phosphomolybdic acid in ethanolic solution. In a limited number of samples showing the presence of free bile acids by thin-layer chromatography, further confirmation of their presence was obtained by means of gas/liquid chromatography. An internal standard of hyocholic acid was added, and the bile acids were converted to the methyl esters of the trifluoroacetates. Gas chromatography was carried out at 230°C with columns containing 1½% QFI on chromosorb W 80/100 (Perkin-Elmer, Ltd). Additional confirmation of the presence of free cholic acid was obtained in some samples by infrared spectroscopy. The extract was streaked on a thin-layer plate, run on solvent system S11 and the area corresponding in position to a developed cholic acid standard run alongside was eluted with acetone. This extract was subjected to infrared spectroscopy (Perkin-Elmer model 137), following incorporation into a potassium bromide disc.

Quantitative measurement of total bile acid concentration was carried out using the 3 alpha-hydroxysteroid dehydrogenase enzyme assay of Iwata and Yamasaki (1964). In the case of samples showing the presence of free bile acids on thin-layer chromatography, the concentration of these and of the conjugated bile acids was measured separately by enzyme assay. They were first separated as streaks by thin-layer chromatography, and then eluted from the plate with methanol, in strips corresponding in position to developed bile acid standards run alongside. The sample itself was shielded during spraying with phosphomolybdic acid. These methods have been shown to be sensitive, accurate, and specific (Northfield, Drasar, and Wright, 1973).

Results

Free (unconjugated) bile acids were demonstrated in postprandial samples from the lower ileum in all subjects studied (fig 1). By contrast, they were absent from the upper jejunum in all subjects. They were found in an increasing proportion of subjects from the level of the lower jejunum downwards. At all levels, free cholic acid was found more frequently than the dihydroxy bile acids. The minimum evidence for identification of free bile acids in each sample was their demonstration by thin-layer chromatography, using two different solvent systems (fig 2). Additional evidence was obtained in some samples from gas/liquid chromatography and from infrared spectroscopy.

The mean postprandial concentration of total bile acids was highest (10 mM) in the upper ileum and lowest (2 mM) in the lower ileum ($P < 0.05$; fig 3). The concentration of free bile acids, on the other hand, progressively increased over the same length of the small intestine, being 0·25 mM in the upper ileum, 0·5 mM in mid ileum, and 1 mM in the lower ileum, at which level thay represented half the total.

In addition to these postprandial samples, fasting samples were also obtained from the region of the duodenojejunal flexure in seven subjects.

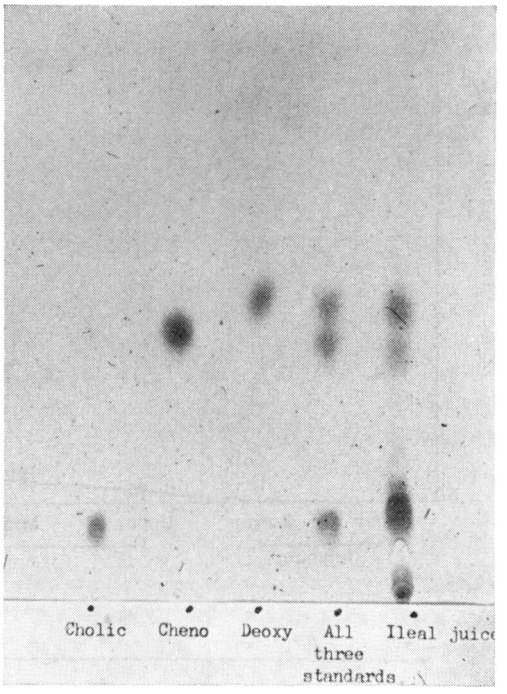

Fig 2 *Thin-layer chromatogram of free bile acid standards and of an ileal extract (trimethyl pentane, ethyl acetate, acetic acid 10:10:2).*

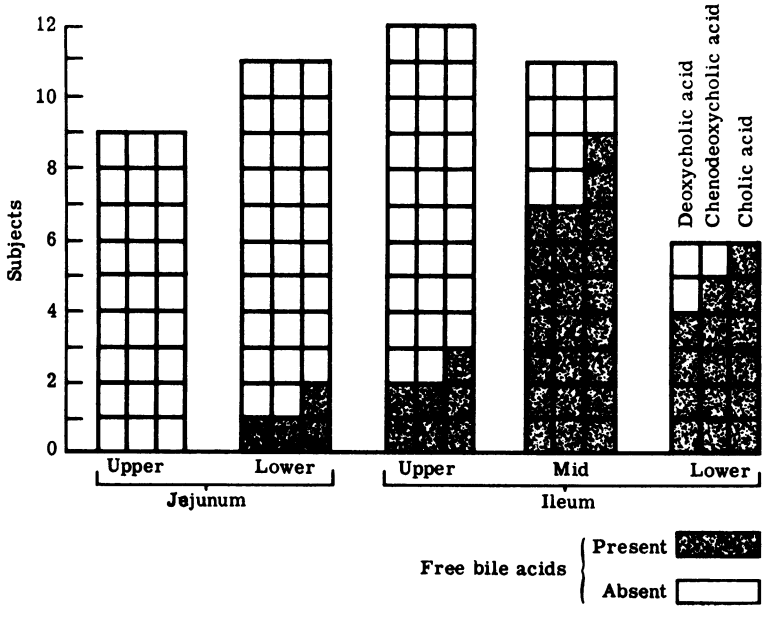

Fig 1 *Distribution of free bile acids down the length of the normal human small intestine.*

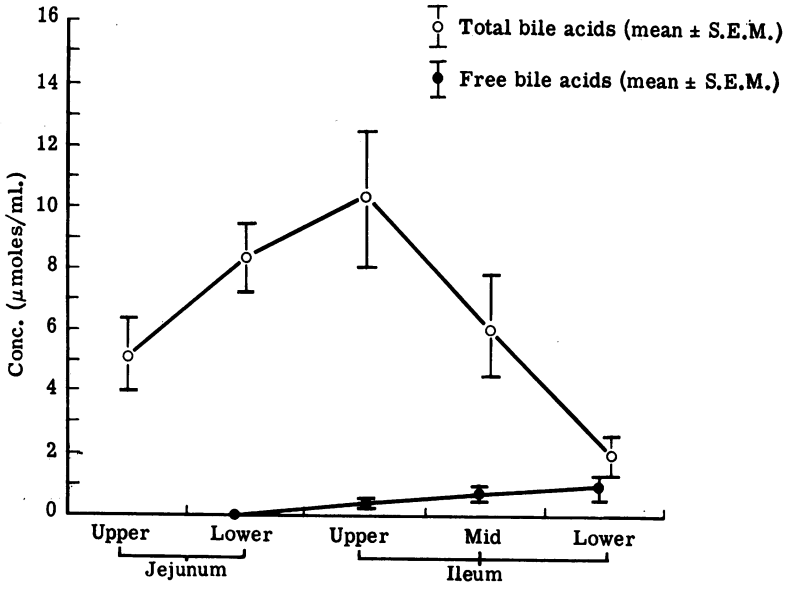

Fig 3 *Concentration of free and total bile acids down the length of the normal human small intestine.*

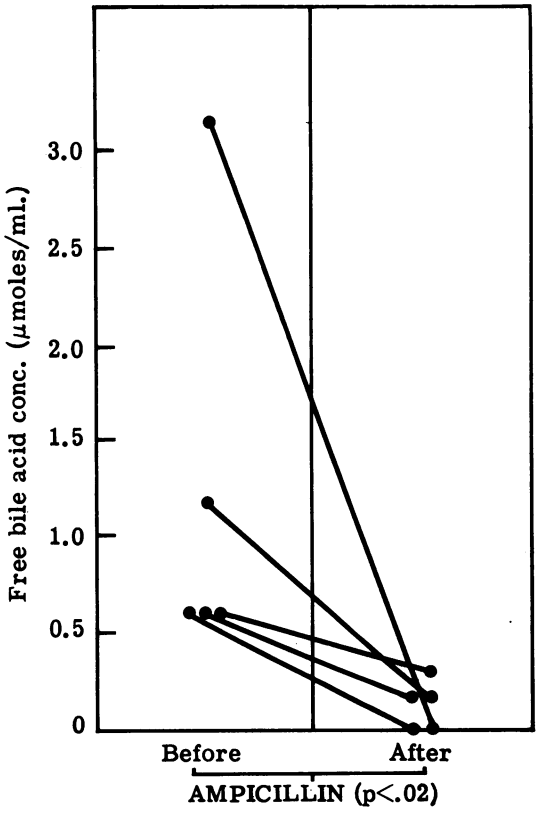

Fig 4 *Ileal concentration of free bile acids before and after ampicillin.*

These gave a mean total bile acid concentration of 3·4 mM. None of these samples contained detectable free bile acids.

The effect of two hours' administration of ampicillin, orally and by tube (500 mg by both routes), was assessed in five subjects with a measurable concentration of free bile acids in ileal juice (fig 4). Care was taken to ensure in each case that the time interval since the last meal was the same for the postampicillin sample as for the preampicillin sample. The mean concentration fell from 1·2 mM to 0·1 mM ($P < 0.02$ by paired t test). By contrast, total bile acid concentration increased from a mean value of 1·0 mM to 7·2 mM following ampicillin ($P < 0.05$) in the same five subjects, together with a sixth subject in whom the sample obtained was of insufficient volume for the separate quantitative measurement of free and conjugated bile acids (fig 5).

Measurements of pH were also carried out on postprandial samples from the same levels of the small intestine. The mean pH was 6·1 in upper and 5·7 in lower jejunal samples. In the ileum there was a progressive rise to 6·7 in upper, 7·2 in mid, and 7·8 in lower ileal samples. The fasting pH was measured in the region of the duodeno-jejunal flexure, and gave a mean value of 3·7.

Discussion

Our findings clearly demonstrate that free (unconjugated) bile acids are present at relatively high concentrations in the lumen of the lower small

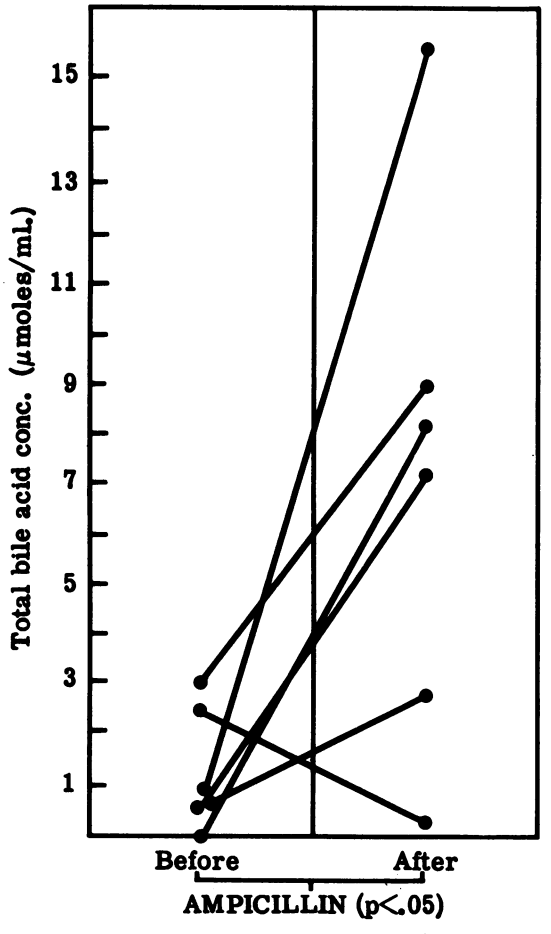

Fig 5 *Ileal concentration of total bile acids before and after ampicillin.*

intestine of subjects with no known gastrointestinal disease. The discrepancy between our findings and those of Sjövall (1959) may be due to different methodology, since Sjövall's analysis was carried out by quantitative paper chromatography, without any prior extraction procedure. On the other hand, our findings are in close agreement with those of Dietschy (1967) in the rat. Like us, he found that the concentration of conjugated bile acids reached a maximum in the mid small intestine, and that unconjugated bile acids were first detected at about the same level.

The fact that the concentration of free bile acids was markedly reduced by ampicillin in our subjects suggests that they resulted from bacterial deconjugation. Bacteriological studies in these subjects, to be described elsewhere (Drasar, Northfield, and Wiggins, unpublished observations), showed the presence of anaerobic bacteria, especially bifidobacteria and bacteroides, throughout the small intestine. The mean concentration rose from 10^3 per ml in the upper jejunum to 10^4/ml in the lower ileum. These bacteria are capable of deconjugating bile acids (Hill and Drasar, 1968), and they are sensitive to ampicillin.

Our study gives no indication of the total quantity of bile acid deconjugated, and the observed concentration of free bile acids is a result of the combined effects of deconjugation and of absorption. Rapid absorption of the free bile acids would result in the observed ratio of free to conjugated bile acids giving a falsely low impression of the total quantity of bile acid being deconjugated.

Our finding that the total bile acid concentration in the ileum increased following ampicillin, despite the decrease in free bile acid concentration, should be interpreted with caution in the absence of a non-absorbable marker. It might, however, indicate impaired absorption due to the reduced concentration of the more rapidly absorbed free bile acids. Dietschy, Salamon, and Siperstein (1966) have presented evidence from experiments in the rat *in vitro* and *in vivo* that absorption is considerably more rapid for free than for conjugated bile acids, in both jejunum and ileum. A similar difference has has been demonstrated in the jejunum for man (Hislop, Hofmann, and Schoenfield, 1967) but ileal absorption was not studied. The free dihydroxy bile acids were more rapidly absorbed than free cholic acid, and this may account for our failure to demonstrate their presence in several samples containing free cholic acid. This rapid absorption of free bile acids is attributed to passive nonionic diffusion, a process that cannot operate for unaggregated conjugated bile acids at the pH of intestinal content, because of their low pK values (approximately 2 to 4). The finding of a pH of more than 7 in the lower ileal content from our subjects and from those of Fordtran and Locklear (1966) might be considered a point against significant absorption of free bile acids by passive nonionic diffusion at this site, since free bile acids have pK values of around 6. There is, however, indirect evidence from studies on drug absorption that the pH of the microclimate at the site of absorption is largely independent of the pH of the bulk solution, and remains between 5 and 6 despite variations in the pH of the perfusing solution (Hogben, Tocco, Brodie, and Schanker, 1959). Although both free and conjugated bile acids can be absorbed by active ileal transport, this process is more effective for the conjugated bile acids (Schiff, Small, and Dietschy, 1972), so that active and passive mechanisms might complement each other if both free and conjugated bile acids were present in the ileal

lumen. Intestinal absorption of bile acids is a remarkably efficient process, involving reabsorption of at least 95% of the bile acids secreted in normal human bile (Gray, Nicholson, and Quincey, 1968). Thus, rapid ileal transport, by a process additional to those available for conjugated bile acids, might have a critical effect on the efficiency of this process, even if the total quantity of free bile acids absorbed was small. Recent studies in man suggest that approximately 15% of bile acids are absorbed in the unconjugated form (Hepner, Hofmann, and Thomas, 1972).

Percy-Robb and Collee (1972) have demonstrated *in vitro* that free bile acids have bactericidal and bacteriostatic effects at concentrations similar to those found by us in the normal ileum, although these effects are more marked at a lower pH than is found in the ileum. They have suggested that our demonstration of the presence of free bile acids in the normal ileum may indicate a homeostatic mechanism controlling retrograde colonization of the small intestine by colonic bacteria.

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